Genetic Relationships Among Tucannon, Touchet, and Walla Walla River Summer Steelhead (*Oncorhynchus mykiss*) Receiving Mitigation Hatchery Fish From Lyons Ferry Hatchery

Scott M. Blankenship*, Maureen P. Small*, Joseph D. Bumgarner†, Mark Schuck†, and Glen Mendel‡

* *Washington Department Fish and Wildlife, Genetics Section, 600 Capitol Way N, Olympia, WA 98501-1091, USA*

† *Washington Department Fish and Wildlife, Snake River Laboratory, 401 S. Cottonwood St., Dayton, WA 99328, USA*

‡ *Washington Department of Fish and Wildlife, Fish Management, 529 W Main St., Dayton, WA 99328, USA*

Summary

Limited information is available on the temporal stability of population allele frequencies. In salmonids, recent empirical studies provide conflicting results regarding the consistency of genetic variation over time within populations. Additionally, since many salmonid populations are of conservation concern and reduced in size, knowledge about effective population size (N_e) and the degree of temporal stability in gene frequencies becomes particularly important as a device for assessing the potential effects of genetic drift. We conduct a temporal analysis of allele frequencies at 14 microsatellite loci for sample collections replicated over a period of eight brood years. We compare the triad of two natural-origin summer steelhead (*Oncorhynchus mykiss*) populations (Tucannon and Touchet rivers) with a single hatchery population (Lyons Ferry Hatchery (LFH) stock) that is used for harvest augmentation within both rivers. We report that allele frequencies for the two natural summer steelhead populations are stable over seven brood years, and the phylogenetic relationships are constant for temporally stratified samples from a single location. In contrast, yearly allele frequency estimates from LFH samples are generally divergent from each other. Evidence suggests that LFH samples may have a lower N_e, as compared to the natural population samples. We also report on several management specific questions, 1) are steelhead caught in the lower and upper Tucannon River trap genetically different, 2) are steelhead that migrate after 1 year in freshwater divergent from those that chose to migrate after 2 or more years in freshwater, and 3) is there evidence for LFH introgression into the Tucannon, Touchet, and Walla Walla Rivers? We find no evidence that steelhead trapped in the lower or upper trap are different genetically. We find no evidence that freshwater age 1 individuals are more related to LFH steelhead, or are genetically different from freshwater age 2-3 steelhead. Based on phylogenetic data and individual assignment analysis we find evidence for LFH introgression into the Tucannon River, but not the Touchet or Walla Walla Rivers. Additionally, there was specific concern for introgression of LFH steelhead into Coppei Creek (Touchet tributary). We found no evidence for LFH introgression to this population. This report also incorporates genetic data from other steelhead studies, which results in the first comparison of lower Columbia River, Walla Walla River, Snake River, and Grand Ronde River steelhead. We report that Kalama River steelhead are approximately twice as differentiated from Tucannon, Touchet, and Walla Walla Rivers (between region $F_{ST} \sim 0.04$) than they are to themselves (Within region $F_{ST} \sim 0.02$). We report that Cougar Creek steelhead are quite differentiated from Tucannon, Touchet, and Walla Walla Rivers (between region $F_{ST} \sim 0.05$). The amount of genetic variance partitioned among groups is similarly different comparing either Rattlesnake Creek or Wallowa stock to the Tucannon, Touchet, and Walla Walla Rivers (between region $F_{ST} \sim 0.02$)

Introduction

Temporal variation in the genetic composition of a population has long been of fundamental interest to evolutionary biologists, since changes in gene frequency over time are the signal of microevolutionary processes and may elucidate the agents responsible for genetic changes in populations (Lessios et al. 1994 and references therein). Although until recently there was a lack of empirical studies reporting genetic diversity estimates based on temporally replicated sampling, there is now an increasing trend in the literature of studies using samples collected over multiple years. This trend is being driven by two factors, 1) concern that biased estimates of population differentiation are being inferred from "snapshot" genetic heterogeneity studies, where samples are collected at single time-points (Waples 1998), and 2) interest in using temporal data to estimate the effective population size (N_e)(Waples 1989), a key parameter in conservation and population biology (Hedgecock et al. 1992). For both these analyses, knowledge about the amount of temporal stability is essential to understanding population trends.

In salmonids, recent empirical studies have provided conflicting results regarding the consistency of within-population genetic variation over time, with both long-term temporal stability and temporal variability observed. There is evidence suggesting that allele frequencies are stable over time, with reports concluding that the temporal variation within a population is minor compared to differences among populations (Banks et al. 2000; Carlsson and Nilsson 2000; Estoup et al. 1998; Hansen et al. 2002; Nielsen et al. 1999; Tessier and Bernatchez 1999). Yet, several recent studies have reported inconsistencies in allele frequency estimates taken from a single population at multiple time periods, with some temporal variation high enough in magnitude to cause erroneous conclusions about population differentiation (Jensen et al. 2005; Laikre et al. 2002; Østergaard et al. 2003; Palm et al. 2003). Analyzing collections from multiple generations to reliably estimate genetic diversities is especially important within a conservation setting, where critical population management decisions are made using population genetic information and population sizes are usually reduced.

Populations with small effective population size (N_e) are more prone to temporal instabilities in gene frequencies and genetic erosions than populations with large N_e (Frankham et al. 2002), since N_e is the main factor mediating any changes in neutral genetic diversity over time caused by genetic drift. Even though temporal variation in gene frequency may not have direct biological significance, stochastic fluctuations in allele frequencies may signify a small N_e, which is a legitimate concern for imperiled populations being impacted by environmental or anthropogenic factors. There are two observations suggesting a potential for reduced N_e in endemic populations of summer steelhead (*Oncorhynchus mykiss*) in western North America. One factor is that census sizes are drastically reduced from historic levels for many steelhead populations (Busby et al. 1997), and N_e is thought to be between 0.10 and 0.33 of the estimated census size (Bartley et al. 1992; Waples pers. comm.). Another factor pertains to the common practice in salmonids for hatchery supplementation programs. Hatchery programs have the potential to alter the N_e of small populations by over-representing certain segments of the population in a subsequent generation, thereby stochastically altering the genetic constitution of the total population (Busack et al. 1997; Ryman and Laikre 1991). A static census size coupled with hatchery supplementation has potential to lower N_e, which then increases the influence of genetic drift and thus temporal fluctuations in allele frequencies. This temporal instability may undermine efforts to document the genetic characteristics of populations and lower the accuracy of inferred genetic relationships between populations. Moreover, the

common assumptions in surveys of genetic diversity that allele frequency estimates are stable over time and do not require temporal study become dubious. N_e depends on a variety of demographic factors and is a difficult quantity to estimate. For salmonids, which exhibit a life history strategy for differential age-at-maturity, each generation of juveniles is produced from multiple cohorts of adults from several previous years. As a result, calculation of N_e in salmonids is complex, and is often reduced to estimating the effective number of breeders (N_b) contributing to a cohort.

Here we describe a genetic analysis of allele frequencies at 14 microsatellite loci for Tucannon, Touchet, and Walla Walla River population samples of summer steelhead collected from 1998 – 2005. We compared the natural summer steelhead populations with Lyons Ferry Hatchery population samples, the source of hatchery mitigation fish. There are no previous genetic studies available comparing these populations, although Waples et al. (1993) found Tucannon River and LFH summer steelhead were differentiated based on allozyme data. Our main objective was to assess the genetic relationships among the natural steelhead samples with that of Lyons Ferry Hatchery. Additionally, we wanted to investigate the genetic relationships of these populations within the broader geographic context of the Snake and Columbia River steelhead populations. There were several secondary objectives of the study due to the complex nature of steelhead, regional reporting requirements, and specific management needs. Box 1 lists a series of questions developed by WDFW Science and Fish Management personnel covered in this report.

Box 1. Questions developed by WDFW Science and Fish Management personnel covered in this report.

Question #1: Are there significant genetic differences between Tucannon, Touchet, or Walla Walla River endemic steelhead stocks?

Question #2: Are there significant genetic differences from any natural steelhead stocks to the Lyons Ferry Hatchery stock?

Question #3: How similar are freshwater Age 1 wild Tucannon River adults to Lyons Ferry Hatchery Stock? Are freshwater Age 2 and Age 3 wild adults different in genetic makeup from freshwater Age 1 fish?

Question #4: How do wild fish collected from lower Tucannon River adult trap compare to wild fish collected at the Tucannon Fish Hatchery trap? Are the lower river collections more similar to Lyons Ferry stock fish?

Question #5: How do the results from this study compare with results and conclusions from Narum et al. (2004)? Is there strong evidence for hatchery introgression into either Walla Walla or Touchet steelhead? Is there evidence of hatchery introgression into Coppei Creek steelhead?

Supplemental Question #1: How do the endemic stocks from the Tucannon, Touchet, and Walla Walla Rivers compare to other steelhead stocks in the Snake or Columbia River basins?

Supplemental Question #2: Given the close similarity between these stocks, how confidently does the data allow us to assign individual fish to the correct location?

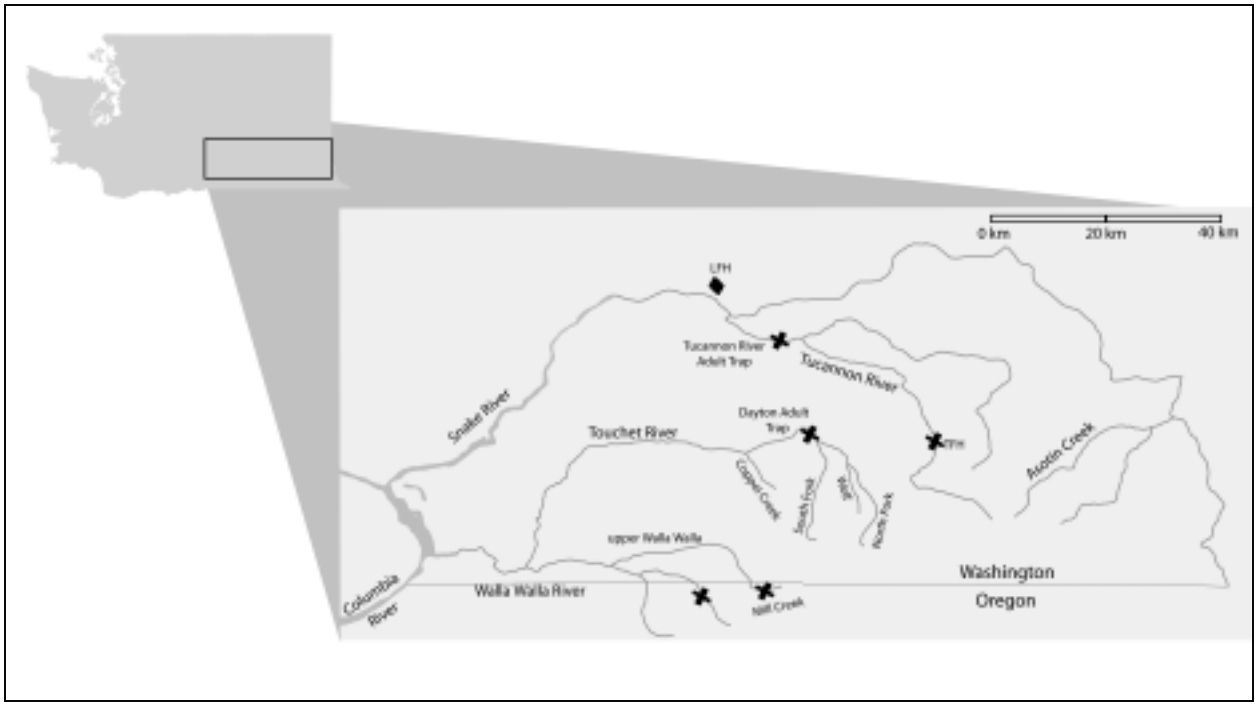

Figure 1. Collection locations for natural Tucannon, Touchet, and Walla Walla River summer steelhead, and the hatchery Lyons Ferry stock. Diamond symbol identifies LFH, X symbols identify trap locations.

Methods and Materials

Tissue collection and DNA Extraction

Natural summer steelhead (*O. mykiss*) individuals used to analyze the temporal stability of allele frequencies were collected 1998 to 2005 from two localities, Tucannon River, a tributary of the Snake River, and Touchet River, a tributary of the Walla Walla River (Figure 1, Table 1). Steelhead adults from the Tucannon River (n=458) were collected at either the lower Tucannon River Adult Trap at river kilometer (rkm) 17.7 or from the Tucannon Fish Hatchery (TFH) Adult Trap at rkm 36 (Figure 1). Steelhead adults from the Touchet River (n=508) were collected at the Dayton Adult Trap, located within the city of Dayton, WA (rkm 87.4). The hatchery sample included in the temporal analysis was from LFH (Figure 1; Table 1). The LFH stock was developed primarily from Wells Hatchery Stock (upper Columbia River) and the Wallowa stock. The Wallowa stock is a composite A and B-run stock that was developed from trapping adult summer steelhead at the lower Snake River dams and reared at Wallowa Hatchery by Oregon Department of Fish and Wildlife. Wells and Wallowa stock fish that returned to LFH during the 1980's and used for broodstock were eventually termed the "LFH stock". Forty-eight adults and 45 juveniles were collected in 1998/1999, and 100 adults were collected each year from 2003-2005, for a total of 393 individuals. Additional samples from the Walla Walla River drainage are shown in Table 1. Although these samples were not included in the temporal analysis, they are listed because they were included in the phylogenetic and N_b analyses. Juvenile samples were collected from five upper Touchet River tributaries in 1999 and 2000: 179 individuals from North Fork Touchet River, 94 individuals from

South Fork Touchet River, 100 individuals from Wolf Fork, 60 individuals from Coppei Creek (a lower Touchet R. tributary), and 59 individuals from Robinson Fork. Fish that escape past the Dayton Adult Trap populate the upper Touchet River, so Touchet River and upper Touchet River samples should be genetically similar. Adult steelhead collections were made in 1998 and 1999 from the Walla Walla River (n=137) and in 1998 from Mill Creek (n=49), a Walla Walla tributary upstream of the Touchet River confluence. Tissue collections were either fin clips or operculum punches, stored immediately in ethanol after collection. DNA was extracted from stored tissue using Nucleospin 96 Tissue following the manufacturer's standard protocol (Macherey-Nagel, Easton, PA, U.S.A.).

Table 1 Within population genetic data analysis summary. N is the number of sampled individuals, A/J is the adult or juvenile life stage, Hz is heterozygosity, LD is linkage disequilibrium.

Sample Collection	N	A/J	Unbiased Hz	Obs. Hz	Allele Richness	Proportion F_{IS}	LD	Bottleneck
98/99 Tucannon River……	36	A	0.809	0.764	13.65	0.056***	0.05	0.30
2000 Tucannon River ……	45	A	0.817	0.757	14.56	0.074***	0.10	0.81
2001 Tucannon River ……	51	A	0.817	0.780	13.99	0.045***	0.03	0.22
2002 Tucannon River ……	45	A	0.826	0.786	14.58	0.049***	0.07	0.39
2003 Tucannon River ……	85	A	0.811	0.727	14.07	0.048***	0.03	0.36
2004 Tucannon River ……	69	A	0.813	0.767	14.39	0.056***	0.08	0.86
2005 Tucannon River ……	127	A	0.815	0.774	14.68	0.049***	0.15	0.54
98/99 Lyons Ferry Hatchery	45	A	0.824	0.796	14.31	0.034**	0.07	0.05*
1999 Lyons Ferry Hatchery…	48	J	0.752	0.702	11.58	0.068***	0.10	0.50
2003 Lyons Ferry Hatchery…	100	A	0.803	0.753	12.53	0.063***	0.23	0.22
2004 Lyons Ferry Hatchery…	100	A	0.806	0.774	13.05	0.040***	0.34	0.24
2005 Lyons Ferry Hatchery…	100	A	0.814	0.793	12.78	0.026***	0.19	0.02*
1999 Touchet River……	33	A	0.819	0.765	14.32	0.067***	0.04	0.24
2000 Touchet River……	30	A	0.812	0.770	13.88	0.052***	0.08	0.90
2001 Touchet River……	116	A	0.811	0.769	13.45	0.052***	0.05	0.33
2002 Touchet River……	85	A	0.811	0.778	13.39	0.042***	0.05	0.24
2003 Touchet River……	73	A	0.803	0.748	13.54	0.069***	0.09	0.15
2004 Touchet River……	96	A	0.816	0.779	13.69	0.046***	0.11	0.17
2005 Touchet River……	75	A	0.823	0.790	13.95	0.040***	0.11	0.58
1999 N.Fork Touchet River…	100	J	0.801	0.774	13.15	0.034***	0.33	0.33
2000 N.Fork Touchet River…	79	J	0.817	0.768	13.21	0.061***	0.15	0.10
1999 S.Fork Touchet River…	94	J	0.811	0.785	13.00	0.033***	0.15	0.30
1999 W.Fork Touchet River	100	J	0.814	0.785	12.82	0.036***	0.35	0.50
2000 Coppei Creek……	60	J	0.793	0.752	12.38	0.052***	0.20	0.24
2000 Robinson Creek……	59	J	0.791	0.769	11.77	0.028***	0.13	0.14
1998 Walla Walla River……	77	A	0.795	0.750	13.40	0.057***	0.03	0.67
1999 Walla Walla River……	60	A	0.810	0.742	13.81	0.084***	0.15	0.22
1998 Mill Creek ……	49	J	0.828	0.759	14.08	0.084***	0.19	0.63

Note. – The α-levels for statistical significance are coded * = 0.05, ** = 0.01, *** = 0.001

Laboratory Analysis

Polymerase chain reaction (PCR) amplification was performed using 14 fluorescently end-labeled microsatellite marker loci, *One* 101, 102, 108 and 114 (Olsen et al. 2000), *Omy* 77 (Morris et al. 1996), *Omy* 1001 and 1011 (Spies et al. 2005), *Omm* 1070, 1128, and 1130 (Rexroad et al. 2001), *Ots* 1 and 3M (Banks et al. 1999), *Ots* 100 (Nelson and Beacham 1999), and *Ots* 103 (Small et al. 1998). PCR reaction volumes were 10 µL, and contained 1 µL 10x PCR buffer (Promega), 1.0 µL $MgCl_2$ (1.5 mM final) (Promega), 0.2 µL 10 mM dNTP mix (Promega), and 0.1 units/µL *Taq* DNA polymerase (Promega). Loci were amplified as part of multiplexed sets, so primer molarities and annealing temperatures varied. Multiplex one had an annealing temperature of 55°C, and used 0.08 Molar (M) *One* 102, 0.07 M *One* 114, and 0.04 M *Ots* 100. Multiplex two had an annealing temperature of 62°C, and used 0.06 M *Omm* 1130, 0.03 M *Omm* 1070, and 0.04 M *Omy* 1011. Multiplex three had an annealing temperature of 55°C, and used 0.04 M *One* 108, 0.011 M *Ots* 103, and 0.021 ML *One* 101. Multiplex four had an annealing temperature of 52°C, and used 0.03 M *Omy* 1001, and 0.025 M *Omm* 1128. Multiplex five had an annealing temperature of 49°C, and used 0.035 M *Ots* 1, 0.03 M *Omy* 77, and 0.02 M *Ots* 3M. All thermal cycling was conducted on a PTC200 thermal cycler (MJ Research) as follows: 95°C (2 min); 30 cycles of 95°C for 30 sec., 30 sec. annealing, and 72°C for 30 sec.; a final 72°C extension and then a 10°C hold. PCR products were visualized by denaturing polyacrylamide gel electrophoresis on an ABI 3730 automated capillary analyzer (Applied Biosystems). Fragment analysis was completed using GeneMapper 3.0 (Applied Biosystems).

Genetic Data Analysis

Assessing within population genetic diversity - Heterozygosity measurements are reported using Nei's (1987) unbiased heterozygosity formula and Hedrick's (1983) formula for observed heterozygosity. Both tests are implemented using the microsatellite toolkit (Park 2001). Allelic richness was calculated using FSTAT version 2.9.3.2 (Goudet 1995). GENEPOP version 3.4 (Raymond and Rousset 1995) was used to assess Hardy-Weinberg equilibrium, where deviations from the neutral expectation of random associations among alleles are calculated using a Markov chain method (5000 iterations in this study) to obtain unbiased estimates of Fisher's exact test. Global estimates of F_{IS} according to Weir and Cockerham (1984) were calculated using FSTAT version 2.9.3.2 (Goudet 1995). Statistical significance at $\alpha = 0.05$ of F_{IS} was adjusted for multiple comparisons. Genotypic linkage disequilibrium was calculated following Weir (1979) using GENETIX version 4.05 (Belkhir et al. 1996). Linkage results are reported as the proportion of pairwise (locus by locus) tests that are significant based on a permutation procedure implemented in GENETIX. Linkage disequilibrium is considered statistically significant if more than 5% of the pairwise tests based on permutation are significant for a sample. To assess if historic changes in population size have caused deviations from mutation-drift equilibrium, we compared observed heterozygosity to that expected under mutation-drift equilibrium, given the observed allele diversity. Excess heterozygosity is expected in populations that have experienced recent size reductions, as rare alleles are lost more rapidly. This test was implemented in the program BOTTLENECK version 1.2.02 (Piry et al. 1999), and statistical significance of the BOTTLENECK result is reported as a

two-tailed Wilcoxon test for heterozygosity excess or deficit, given a two-phase microsatellite mutation model. P-value significance was not adjusted for multiple tests.

Temporal analysis of allele frequencies - Within a location, temporal samples were compared using Friedman's method for randomized blocks (Sokal and Rohlf 3rd edition pg. 440), a non-parametric analysis of variance (ANOVA) without replication. The total number of alleles, observed heterozygosity, and unbiased heterozygosity were used as blocks, with the collection year as treatment effect (Appendix 1). The null hypothesis for this test is that there is no year effect. The temporal stability of allele frequencies was assessed by the genetic differentiation randomization chi-square test implemented in FSTAT version 2.9.3.2 (Goudet 1995). Alleles were randomized between samples (i.e. genic test).

Effective population size (N_e) – Estimates of the effective population size were obtained using two methods, a multi-sample temporal method (Waples 1990) on consecutive cohorts of steelhead and a single sample linkage disequilibrium method on upper Touchet River juvenile samples. Combining population samples with age data from scale analysis generated cohort samples. Only cohorts samples with greater than 20 individuals were used in the temporal method analysis. For the temporal method, $\hat{F}$ (standardized variance in allele frequency) is calculated according to Pollack (1983). The parameter b is calculated analytically from age structure information and the number of years between samples (Tajima 1992). The age-at-maturity information required to calculate b was obtained from the cohort data. Waples (1990) developed a method to estimate the effective number of breeder (N_b) from $\hat{F}$ that incorporates the Pacific salmon life history:

$$\hat{N}_{b(i,j)} = \frac{b}{2\,(\hat{F} - 1/\hat{S}_{i,j})}$$

The Waples (1990) temporal method has been updated by Waples et al. (2007). Consecutive cohort samples are analyzed to estimate the pairwise N_b ($\hat{N}_{b(i,j)}$). Various $\hat{N}_{b(i,j)}$ will not have the same information content (sample size, alleles), so pairwise estimates are weighted by the reciprocal variance of the global estimate of N_b ($\tilde{N}_b$) (i.e. harmonic mean of all $\hat{N}_{b(i,j)}$). $\tilde{N}_b$ is the estimate of the effective population size (N_e). SALMONNb (Waples et al. 2007) was used to calculate ($\tilde{N}_b$) (i.e. N_e). A single sample method was used to estimate N_b from the Touchet River juvenile samples. Juvenile samples may be used for N_b calculations, although estimates are not directly comparable to estimates made using temporal methods, as juvenile samples estimate N_b for the parental generation only (Waples 2005). The linkage disequilibrium method was used to estimate N_b for each juvenile sample from the upper Touchet River. This method uses the mean squared correlation of allele frequencies at different gene loci (Bartley et al. 1992; Campton 1987; Waples 1991). Estimates of the linkage disequilibrium method were calculated using the software NEESTIMATOR (Peel et al. 2004).

Among population genetic differentiation - Population structure was investigated using pairwise estimates of F_{ST} and within an analysis of molecular variance framework (AMOVA). Multi-locus estimates of pairwise F_{ST}, estimated by a "weighted" analysis of variance (Weir and Cockerham, 1984), were calculated using GENEPOP version 3.4 (Raymond and Rousset 1995). To determine if

the F_{ST} estimates were statistically different from zero, 1000 permutations were implemented in GENETIX version 4.05 (Belkhir et al.1996). The hierarchical AMOVA partitioned the total variance into covariance components due to intra-individual, inter-individual, inter-population, and inter-group differences (Weir and Cockerham, 1984). The covariance components are used to compute fixation indices (i.e. probability of identity by descent) in terms of coalescent times. The significance of the fixation indices was tested using a non-parametric permutation approach described in Excoffier et al. (1992). After each permutation round, 20,000 in total, all variance statistics are recomputed to get their null distribution. ARLEQUIN 3.01 (Excoffier et al. 2005) was used to conduct the AMOVA. The structure of the AMOVA was the same as the temporal analysis of allele frequencies, and defined three groups, Tucannon River, Touchet River, and LFH. All temporally replicated samples were analyzed within each group.

Genetic distances were calculated using the program GENDIST (Phylip 3.6, Felsenstein 2005), using the Cavalli-Sforza's chord measure (Cavalli-Sforza and Edwards, 1967). The neighbor-joining algorithm was used to construct trees (Phylip 3.6, Felsenstein 2005). The robustness of the population tree topology was assessed using 1000 bootstrap datasets of the above genetic distances and the program CONSENSE (Phylip 3.6, Felsenstein 2005).

Individual assignment – A population baseline file containing 1,948 individuals was constructed, with samples subdivided based on genetic similarity into four population categories, Tucannon, LFH, Touchet, and Walla Walla. All individuals in baseline had data for 10 or more loci. Individual steelhead were assigned to their most likely population of origin based on the partial Bayesian criteria of Rannala and Mountian (1997), using a "jack-knife" procedure, where each individual to be assigned was removed from the baseline prior to the calculation of population likelihoods. All tests were implemented using GENECLASS2 software (Piry et al. 2004). A LOD score assessed the quality of each assignment. The LOD statistic was manually constructed using the likelihood rank scores from each GENECLASS2 assignment, with the odds ratio having the form of "most likely" divided by "second most likely". An individual was classified as unassigned if the assignment LOD < 1. In addition to requiring a minimum LOD score for assignment, the probability of each assignment was assessed using the simulation procedure of Paetkau et al. (2004). The simulation was used to exclude a population from consideration for the assignment of an individual. If the probability of any assignment did not fall within the expected 95% confidence interval derived by the simulation, the rank-based assignment was not allowed to that population irrespective of LOD score. The results reported are the proportions of individuals assigned to each population category, given that the assignment LOD was greater than one and that the individual's likelihood resided within the 95% confidence interval for the estimated population of origin.

Results

Microsatellite diversity within populations - Substantial genetic diversity was observed within populations, with unbiased heterozygosity estimates, over all loci, ranging from a low of 0.752 (1999 LFH) to 0.828 (1998 Mill Creek) (Table 1). Mean allele richness over all populations and loci was 13.50, with allele richness ranging from a low of 11.58 (1999 LFH) to a high of 14.68 (2005 Tucannon) (Table 1). The number of alleles sampled per locus was standardized to the smallest sample size of complete genotypes (N=28, 56 alleles) using a rarefaction method, although the mean sample size was much larger (N=73). Departures from expected random mating genotypic

proportions, quantified as statistically significant heterozygote deficiencies (F_{IS}), were observed for all populations (Table 1). Values ranged from a high of 7.4% deviation from expectation for the 2000 Tucannon River sample, to a low of 2.6% deviation for the 2005 LFH sample (Table 1). Significant linkage disequilibrium was detected for 21 of the 28 samples (Table 1). Results for tests of mutation-drift equilibrium (BOTTLENECK) are shown in Table 1. The BOTTLENECK results reported are the p-values for the null hypothesis of equilibrium, with significant deviations from the null expectation observed for the 98/99 LFH (p= 0.05) and the 2005 LFH (p= 0.02) samples. All other population samples were consistent with mutation-drift equilibrium based on the comparison between observed allelic diversity and expected heterozygosity.

Temporal analysis of allele frequencies - The null hypothesis of no year effect was rejected for five out of 42 ANOVA tests using Friedman's method for randomized blocks. A significant year effect was seen at *Ots* 100 for Tucannon River, *Ots* 1 and *Omy* 1001 for LFH, and *Ots* 1 and *Ots 103* for Touchet River samples. P-values for genic differentiation tests (within region) are shown in Table 2, with pairwise comparisons of allele frequencies conducted separately for collections where stratified temporal samples were available. (A p-value of 0.0001 is significant at alpha=0.05 after correction for multiple tests). Allele frequencies for all Tucannon River samples, except one pairwise comparison, were statistically equivalent (Table 2). The comparison between 2003 and 2005 Tucannon samples were differentiated based on the chi-square test. Regarding LFH samples, all samples were largely differentiated. The 98/99 and 2004 sample comparison was the only pairwise test that was statistically equivalent (Table 2). For the Touchet River samples, 2001 and 2002 samples were differentiated from the 2004 sample. The allele frequencies for the 2000 Touchet sample are not equivalent to all other Touchet River samples. Regarding the upper Touchet River tributary samples, all samples are statistically different (Table 2). Both Walla Walla samples are statistically equivalent, but the p-value is low. The Walla Walla samples are statistically different from the Mill Creek sample.

P-values for genic differentiation tests (between region) are shown in Table 3, with pairwise comparisons of allele frequencies conducted separately for collections where stratified temporal samples were available. In general, between region allele frequency comparisons are statistically different. The 98/99 LFH sample was statistically equivalent to all the Tucannon River samples. There was also some similarity between the 2000 Touchet sample and the Tucannon samples. The 1999 Touchet sample was similar to the upper Touchet juvenile samples, and all Walla Walla River samples.

Table 2 Genetic differentiation. Values for within population pairwise tests are shown for Tucannon, Touchet, Lyons Ferry Hatchery, and Walla Walla collections. Above the diagonal are p-values for pairwise tests of allelic differentiation. Below the diagonal are pairwise estimates of F_{ST}. Statistically significant pairwise F_{ST} estimates are bolded.

	98/99Tuc	00Tuc	01Tuc	02Tuc	03Tuc	04Tuc	05Tuc
98/99Tucannon	-	0.4082	0.5291	0.1607	0.2357	0.0433	0.0024
00 Tucannon…	0.001	-	0.2341	0.7335	0.1665	0.0597	0.0126
01 Tucannon…	0.000	0.001	-	0.4376	0.0448	0.2612	0.0373
02 Tucannon…	0.003	-0.001	0.000	-	0.5320	0.6660	0.0217
03 Tucannon…	0.001	0.001	0.000	-0.001	-	0.0315	0.0001
04 Tucannon…	**0.004**	0.001	0.001	-0.001	0.001	-	0.0851
05 Tucannon…	**0.004**	**0.004**	0.001	**0.003**	**0.002**	**0.002**	-

	98/99LFH	99LFH	02LFH	04LFH	05LFH
98/99LFH	-	0.0001	0.0001	0.0004	0.0001
99LFH …	**0.037**	-	0.0001	0.0001	0.0001
02LFH …	**0.004**	**0.040**	-	0.0001	0.0001
04LFH …	0.002	**0.039**	**0.006**	-	0.0001
05LFH …	0.002	**0.036**	**0.008**	**0.007**	-

	99Tou	00Tou	01Tou	02Tou	03Tou	04Tou	05Tou
99 Touchet	-	0.0020	0.0050	0.0003	0.0152	0.0052	0.0045
00 Touchet	**0.026**	-	0.0001	0.0001	0.0001	0.0001	0.0001
01 Touchet	0.001	**0.027**	-	0.0114	0.1470	0.0001	0.0049
02 Touchet	0.001	**0.032**	**0.002**	-	0.5279	0.0001	0.0217
03 Touchet	0.003	**0.029**	**0.002**	0.000	-	0.0004	0.0163
04 Touchet	0.001	**0.029**	0.002	**0.002**	**0.003**	-	0.0023
05 Touchet	0.002	**0.030**	0.001	**0.003**	**0.004**	**0.002**	-

	99NFTou	00NFTou	99SFTou	99WFTou	00Copp	00RobTou
99NFTou	-	0.0001	0.0001	0.0001	0.0001	0.0001
00NFTou	**0.010**	-	0.0001	0.0001	0.0001	0.0001
99SFTou	**0.011**	**0.011**	-	0.0001	0.0001	0.0001
99WFTou	**0.009**	**0.011**	**0.009**	-	0.0001	0.0001
00Coppei	**0.015**	**0.021**	**0.016**	**0.018**	-	0.0001
00Robinson	**0.016**	**0.014**	**0.013**	**0.011**	**0.021**	-

	98 Walla	99 Walla	98 Mill
98 Walla	-	0.0008	0.0001
99 Walla	0.002	-	0.0001
98 Mill	**0.007**	**0.006**	-

Table 3 Genetic differentiation. P-values for between population pairwise tests of allelic differentiation are shown for Tucannon, Touchet, Lyons Ferry Hatchery, and Walla Walla collections.

	98/99 LFH	99 LFH	03 LFH	04 LFH	05 LFH	99 Tou	00 Tou	01 Tou	02 Tou	03 Tou	04 Tou	05 Tou	98 Walla	99 Walla	98 Mill
98/99 Tuc	0.1978	*	*	*	*	*	*	*	0.0004	*	*	*	*	*	*
2000 Tuc	0.6952	*	*	*	*	0.0216	*	*	*	*	*	*	*	*	*
2001 Tuc	0.1947	*	*	*	*	*	*	*	*	*	*	*	*	*	*
2002 Tuc	0.6204	*	*	*	*	0.0034	*	*	*	*	*	*	*	*	*
2003 Tuc	0.4730	*	*	*	*	0.0003	*	*	*	*	*	*	*	*	*
2004 Tuc	0.4855	*	*	*	*	*	*	*	*	*	*	*	*	*	*
2005 Tuc	0.0009	*	*	*	*	0.0003	*	*	*	*	*	*	*	*	*

	99 Tou	00 Tou	01 Tou	02 Tou	03 Tou	04 Tou	05 Tou	98 Walla	99 Walla	98 Mill
98/99 LFH	*	*	*	*	*	*	*	*	*	*
1999 LFH	*	*	*	*	*	*	*	*	*	*
2003 LFH	*	*	*	*	*	*	*	*	*	*
2004 LFH	*	*	*	*	*	*	*	*	*	*
2005 LFH	*	*	*	*	*	*	*	*	*	*

	98 Walla	99 Walla	98 Mill
99TouA	0.0012	0.0492	0.0036
00TouA	*	0.0004	*
01TouA	*	*	*
02TouA	*	*	*
03TouA	*	*	*
04TouA	*	*	*
05TouA	*	*	*

	98 - 05 Tuc	98 - 05 LFH	99 Tou	00 Tou	01 Tou	02 Tou	03 Tou	04 Tou	05 Tou	98 Walla	99 Walla	98 Mill
99NFTouJ	*	*	0.0075	*	*	*	*	*	*	*	*	*
00NFTouJ	*	*	0.0774	0.0003	*	*	*	*	*	*	*	*
99SFTouJ	*	*	0.0489	*	*	*	*	*	*	*	*	*
99WFTouJ	*	*	0.1852	*	*	*	*	*	*	*	*	*
00CoppJ	*	*	*	*	*	*	*	*	*	*	*	*
00RobTouJ	*	*	0.0004	*	*	*	*	*	*	*	*	*

Note - * denotes a p-value of 0.0001 or less. Tuc = Tucannon, LFH = Lyons Ferry Hatchery, Tou = Touchet, Walla = Walla Walla

Microsatellite diversity among populations - Significant heterogeneity in allele frequencies was observed among populations, although the variance attributed to population subdivision was small. The global F_{ST} value was 0.013 (+/- 0.004). Between watersheds (termed group in AMOVA), the mean pairwise F_{ST} estimates were, 0.010 Tucannon River v. Touchet River (0.006 when 2000 Touchet sample excluded), 0.011 Tucannon River v. LFH (0.006 when 1999 LFH juvenile sample excluded), and 0.023 LFH v. Touchet River (0.012 excluding both 1999 LFH and 2000 Touchet).

The proportion of variation attributed to the among-group AMOVA component was 0.44% (Table 4). Additionally, the proportion of variance attributed to among-population differences within groups, 0.86% in Table 4, was similar to that observed for among-group differences. Correspondingly, the mean pairwise F_{ST} estimates from temporally replicated samples were 0.001 for Tucannon River, 0.010 for Touchet River (0.002 with 2000 Touchet sample excluded), and 0.018 for LFH (0.005 when 1999 LFH juvenile sample excluded).

Table 4 Global AMOVA results as a weighted average over loci.

Source of variation	Variance components	Percentage variation
Among groups	0.0258	0.44
Among populations within groups	0.0500	0.86
Among individuals within populations	0.4922	8.47
Within individuals	5.2436	90.23

Effective population size – Estimates of effective number of breeder (N_b) derived from Waples (1990) temporal method are shown in Table 5-7. For the Tucannon River samples, cohorts from 1997 – 2002 were used (Table 5). From scale analysis for all Tucannon samples, 6% of individuals were age 2, 48% were age 3, 43% were age 4, and 3% were age 5. Those percentages were used as the population age-at-maturity information to calculate b. The harmonic mean of all pairwise estimates of N_b ($\widetilde{N}_b$) was 222.7. This estimate is the contemporary N_e for Tucannon River. For the Touchet River samples, cohorts from 1996 – 2002 were used (Table 6). From scale analysis for all Touchet samples, 2% of individuals were age 2, 53% were age 3, 39% were age 4, and 6% were age 5. Those percentages were used as the population age-at-maturity information to calculate b. The harmonic mean of all pairwise estimates of N_b ($\widetilde{N}_b$) was 173.8. This estimate is the contemporary N_e for Touchet River. For the LFH samples, cohorts from 2000 – 2002 were used (Table 7). From scale analysis for all LFH samples, 85% were age 3, 15% were age 4. Those percentages were used as the population age-at-maturity information to calculate b. The harmonic mean of all pairwise estimates of N_b ($\widetilde{N}_b$) was 144.4. This estimate is the contemporary N_e for LFH stock.

The linkage disequilibrium method was used to estimate N_b for each juvenile sample from the upper Touchet River. The estimates were of similar magnitude as the pairwise estimates derived from the

temporal method. The N_b estimates ranged from a low of 81.2 for 2000 Coppei Creek to a value of 206.2 for 2000 NF Touchet.

Table 5 Summary of output from program SALMONNb and data for six consecutive years of summer steelhead samples from Tucannon River. For each pairwise comparison of samples *i* and *j*, $\widetilde{S}$ is the harmonic mean sample size, *n* is the number of independent alleles used in the comparison, $\hat{N}_{b(i,j)}$ are the pairwise estimates of N_b, and Var $[\hat{N}_{b(i,j)}]$ is the variance of $\hat{N}_{b(i,j)}$. $\widetilde{N}_b$ is the harmonic mean of the $N_{b(i,j)}$. Alleles with a frequency below 0.05 were excluded from the analysis to reduce potential bias.

Year	1997	1998	1999	2000	2001	2002
Pairwise $\widetilde{S}$ (above diagonal) and *n* (below diagonal):						
1997		31.6	39.7	35.4	43.4	32.5
1998	87		47.1	41.2	52.5	37.4
1999	87	85		56.1	79.5	49.3
2000	84	82	87		63.9	42.9
2001	90	85	87	86		55.3
2002	84	89	87	90	85	
Pairwise $\hat{N}_{b(i,j)}$ (above diagonal) and Var $[\hat{N}_{b(i,j)}]$ (below diagonal):						
1997		475.3	152.3	256.5	98.2	155
1998	53225		infinity	430.7	437.3	100.7
1999	31638	28082		228.6	1307.8	92.5
2000	97677	31602	20881		970	253.4
2001	55514	48584	11164	17370		302.8
2002	43823	72659	52731	27012	21901	
$\widetilde{N}_b$	= 222.7					

Table 6 Summary of output from program SALMONNb and data for seven consecutive years of summer steelhead samples from Touchet River. For each pairwise comparison of samples *i* and *j*, $\tilde{S}$ is the harmonic mean sample size, *n* is the number of independent alleles used in the comparison, $\hat{N}_{b(i,j)}$ are the pairwise estimates of N_b, and Var $[\hat{N}_{b(i,j)}]$ is the variance of $\hat{N}_{b(i,j)}$. $\tilde{N}_b$ is the harmonic mean of the $N_{b(i,j)}$. Alleles with a frequency below 0.05 were excluded from the analysis to reduce potential bias.

Year	1996	1997	1998	1999	2000	2001	2002
Pairwise $\tilde{S}$ (above diagonal) and *n* (below diagonal):							
1996		31.2	38.7	40.5	32.2	32.7	26.1
1997	82		50.3	53.4	39.9	40.7	30.9
1998	77	81		80.2	53.1	54.6	38.3
1999	79	81	77		56.6	58.3	40.1
2000	76	80	86	82		42.5	32
2001	83	82	85	79	88		32.5
2002	75	81	83	85	87	86	
Pairwise $\hat{N}_{b(i,j)}$ (above diagonal) and Var $[\hat{N}_{b(i,j)}]$ (below diagonal):							
1996		infinity	298.7	146.8	infinity	509.6	176.2
1997	21986		83.6	89.1	82.9	63.3	101.7
1998	13996	10557		632.8	1052.1	124.4	166.1
1999	35422	8289	5864		410.9	256.9	58.4
2000	38274	35865	7868	8804		infinity	185.6
2001	17009	23905	19989	7538	12509		212.1
2002	35984	19100	26129	33484	16690	19587	
$\tilde{N}_b$	= 173.8						

Table 7 Summary of output from program SALMONNb and data for three consecutive years of summer steelhead samples from Lyons Ferry Hatchery. For each pairwise comparison of samples *i* and *j*, $\tilde{S}$ is the harmonic mean sample size, *n* is the number of independent alleles used in the comparison, $\hat{N}_{b(i,j)}$ are the pairwise estimates of N_b, and Var $[\hat{N}_{b(i,j)}]$ is the variance of $\hat{N}_{b(i,j)}$. $\tilde{N}_b$ is the harmonic mean of the $N_{b(i,j)}$. Alleles with a frequency below 0.05 were excluded from the analysis to reduce potential bias.

Year	2000	2001	2002
Pairwise $\tilde{S}$ (above diagonal) and *n* (below diagonal):			
2000		96.3	84.2
2001	85		92.2
2002	91	90	
Pairwise $\hat{N}_{b(i,j)}$ (above diagonal) and Var $[\hat{N}_{b(i,j)}]$ (below diagonal):			
2000		225.2	114.1
2001	1194.2		132.1
2002	1172.3	1164.3	
$\tilde{N}_b$	= 144.4		

Table 8 Estimates of the effective number of breeders (N_b) for the parental cohorts contributing to juvenile steelhead samples from the Upper Touchet River. Single samples were analyzed using the linkage disequilibrium method (Bartley et al. 1992; Waples 1991).

Sample	N_b	Confidence Interval
1999 NF Touchet	118.1	(107.6 – 130.4)
2000 NF Touchet	206.2	(172.3 – 254.9)
1999 SF Touchet	157.7	(139.0 – 181.2)
1999 Wolf F Touchet	100.8	(92.7 – 110.1)
2000 Coppei Creek	81.2	(71.5 – 93.3)
2000 Robinson Creek	93.8	(81.0 – 110.6)

Genetic distance analysis - Considering the Tucannon and Touchet rivers samples and LFH, analysis of genetic distances among populations revealed two distinct clusters of population samples (Figure 2) with strong bootstrap support (98.5%) for a division between Touchet River and a grouping containing Tucannon River and LFH. Within the Tucannon group, the branch containing LFH had lower bootstrap support (66%) (Figure 2), and contained all but the 98/99 collection.

The analysis was extended to include samples from the upper tributaries of the Touchet River and samples from the Walla Walla River to give a wider geographic perspective. When additional samples were included in the analysis, the same basic genetic relationships remained (Figure 3). The Tucannon River and LFH were distinct from all Walla Walla River populations. Within the Walla Walla River, the Touchet River samples from 2001-2005 formed a distinct cluster within the tree, separated from the remaining samples from the Walla Walla River (Figure 3). In the population tree, the 1999 Touchet sample is placed within the upper Touchet tributary samples, and the 2000 Touchet River sample pairs with the 2000 North Fork Touchet River sample. Additionally, there was bootstrap support for a population cluster containing the Upper Walla Walla River and Mill Creek samples.

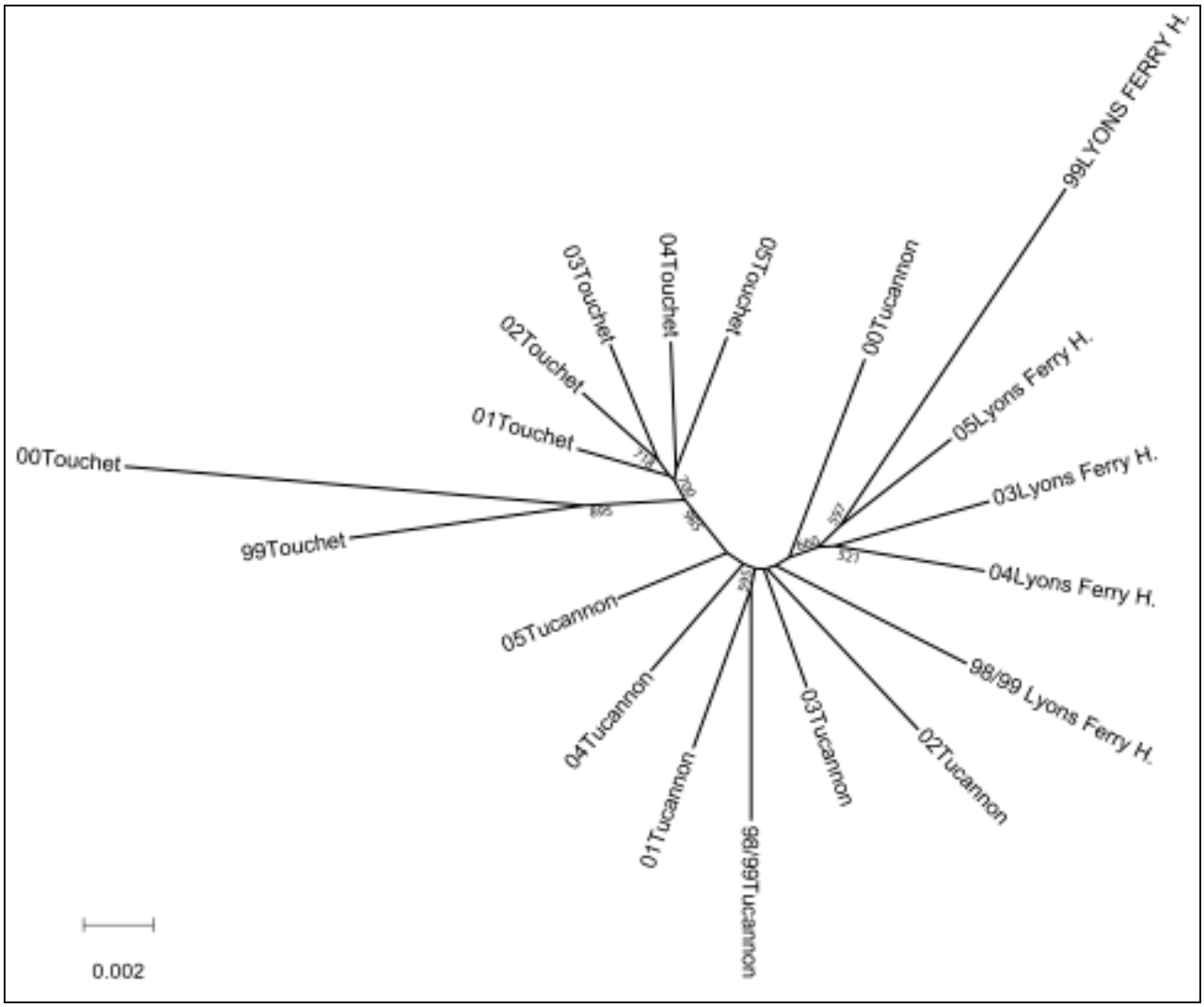

Figure 2. Chord-distance tree for temporally stratified adult samples. Node support numbers are values from bootstrap analysis (1000 bootstraps). Note: only 1999 LFH samples were from juveniles.

Figure 3. Chord distance tree that includes temporally stratified samples (from Figure 2), plus samples from Touchet River tributaries, Mill Creek, and Walla Walla River. Sample labels with all letters capitalized are juvenile samples. Node support numbers are values from bootstrap analysis (1000 bootstraps).

Individual assignment – Assignment proportions are shown in Table 9. The Tucannon steelhead sample had the lowest self-assignment proportion, 29%, and the highest number of unassigned individuals, 43%. Additionally, 14% assigned to LFH, 9% assigned to the Touchet and 5% assigned to the Upper Walla Walla. The LFH had a 46% self-assignment rate, approximately 10% assignment to Tucannon and Touchet, and 1% assignment to Walla Walla. The Touchet sample had 53% self-assignment, 6% assignment to Tucannon, 5% assignment to LFH, and 5% assignment to Walla Walla. The Walla Walla sample had the highest self-assignment rate, 56%, the fewest number of individuals assigning to LFH, 1%, and the lowest number of unassigned fish, 27%.

Table 9 Individual assignment results reported are the proportions of individuals assigned to each population category, given the assignment LOD was greater than one and the individual's likelihood resided within the 95% confidence interval for the estimated population of origin.

	N	Tucannon	LFH	Touchet	Walla Walla	Unassigned
Tucannon River ……	451	0.29	0.14	0.09	0.05	0.43
Lyons Ferry Hatchery	333	0.10	0.46	0.13	0.01	0.31
Touchet River……	987	0.06	0.05	0.53	0.05	0.30
Walla Walla ……	177	0.04	0.01	0.12	0.56	0.27

Discussion

Results interpreted related to questions from Box 1

Question #1 - Are there significant genetic differences between Tucannon, Touchet, or Walla Walla River endemic steelhead stocks?

Data from both the genic differentiation tests (Table 2 and Table 3) and genetic distance analysis (Figures 2, 3, and 4) address this question. We report that allele frequencies for two natural summer steelhead populations (Tucannon and Touchet Rivers) were stable over seven brood years (Table 2). Therefore, allele frequencies for population samples from a single location (e.g. Tucannon River samples) are statistically equivalent from year to year. With the exception of the pairwise comparison between the 2003 and the 2005 samples, allele frequency estimates from eight consecutive years of Tucannon River collections were statistically equivalent (Table 2). For the Touchet River collections, six of the seven sample years were statistically equivalent and the 2000 sample year (N=30 adults) appears to be anomalous (Table 2). This same observation holds for the Walla Walla samples, where the 1998 Walla Walla sample is not statistically differentiated from the 1999 Walla Walla, although the p-value is low (Table 2). In contrast, most of the between population genic differentiation tests are statistically different. Therefore, the Tucannon, Touchet, and Walla Walla Rivers are genetically distinct (Table 3), although the divergence is slight (Table

4). The genetic distance dendrograms (Figures 2 – 4) also support the conclusion that the Tucannon, Touchet and Walla Walla Rivers are genetically distinct, since samples from the same population cluster together on the tree.

Question #2 - Are there significant genetic differences from any natural steelhead stocks to the Lyons Ferry Hatchery stock?

Contrary to the results for natural population samples, the allele frequency estimates for LFH samples were temporally divergent (Table 2). The only pairwise allele frequency comparison from LFH that was statistically equivalent was between the 98/99 and 2004 samples, although the p-value was low; all other sample comparisons were divergent. Additionally, the 98/99 LFH sample was indistinguishable from the Tucannon River natural samples (Table 3). This observation implies that the 98/99 LFH adult sample, comprised of marked hatchery fish collected at the TFH trap, either had allele frequencies similar to the natural Tucannon River sample by chance, or the natural sample contained many steelhead with hatchery ancestry that year. The later explanation is most likely, since LFH stock hatchery fish were the dominant returns to the area above the hatchery in the years from which the natural origin fish originated. In addition to LFH samples having statistically different allele frequencies by year, Table 3 shows that LFH (with the exception of the 98/99 LFH collection) is divergent from Tucannon, Touchet, and Walla Walla River samples.

Question #3 - How similar are freshwater Age 1 wild Tucannon River adults to Lyons Ferry Hatchery Stock? Are freshwater Age 2 and Age 3 wild adults different in genetic makeup than freshwater Age 1 fish?

During 2000 – 2005, 47 natural origin steelhead were sampled that were freshwater age 1 and N=288 were freshwater age 2 or 3. We tested equivalency of allele frequencies between the two sample sets using FSTAT (i.e. allelic differentiation). The allele frequencies were statistically equivalent (p-value 0.55). Additionally the estimated pairwise F_{ST} was negligible between the two sample groups (F_{ST} = 0.0008). These results suggest there is no difference between the steelhead choosing to emigrate after one year in freshwater versus two or more.

Question #4 - How do wild fish collected from lower Tucannon River adult trap compare to wild fish collected at the Tucannon Fish Hatchery trap? Are the lower river collections more similar to Lyons Ferry stock fish?

There were only two sample years that steelhead were collected from both upper and lower Tucannon River traps. For the 2003 sample year, 16 steelhead were sampled at the TFH trap and 65 steelhead were sampled at the lower Tucannon River adult trap. For the 2004 sample year, 8 steelhead were sampled at the TFH trap and 47 steelhead were sampled at the lower Tucannon River adult trap. The substantially different sample sizes for the trapping locations limits the statistical power of comparisons. We subdivided genetic data by trapping location, combining all Tucannon River samples (years 1998/1999 – 2005) collected from the lower Tucannon River adult trap (N=347) and combining steelhead samples from 2003 and 2004 collected from the TFH trap (N=24),

and tested equivalency of allele frequencies between the two sample sets using FSTAT (i.e. allelic differentiation). The allele frequencies were statistically equivalent (p-value 0.20). Additionally the pairwise F_{ST} estimated was negligible between the two sample groups. These results suggest there is no difference between the steelhead trapped in the upper or lower Tucannon River traps.

Question #5 - How do the results from this study compare with results and conclusions from Narum et al. 2004? Is there strong evidence for hatchery introgression into either Walla Walla or Touchet steelhead? Is there evidence of hatchery introgression into Coppei Creek steelhead?

We found that Walla Walla River samples were significantly different genetically from Touchet River samples. Narum et al. (2004) reported this result as well. Narum et al. (2004) largely focuses on differentiation between resident and anadromous forms of steelhead within the same stream. Our study did not include resident rainbow populations, so it is difficult to comment on Narum's results regarding resident rainbow; however one sample from our study has mixed ancestry (1998 Mill Creek). That sample likely includes both juvenile steelhead and large resident rainbows from Mill Creek. The 1998 Mill Creek sample is statistically different from both Walla Walla River steelhead samples. Narum et al. (2004) reported heterozygote deficits in the Touchet River. Heterozygote deficit could be the result of population admixture within a sample or an artifact resulting from variable age-at-maturity. We observed heterozygote deficit in the Tucannon, Touchet, and Walla Walla Rivers. The deviations were approximately 5%, and this amount of deficit is typical for salmon populations. Due to low levels of linkage disequilibrium observed in the Tucannon and Touchet River samples, admixture in these population samples is unlikely. The 1999 Walla Walla sample shows elevated levels of linkage disequilibrium compared to the 1998 sample. It is possible the 1999 Walla Walla sample contains resident rainbow (note: samples were all adult steelhead >20 inches in length), but the genetic distance analysis shows the Walla Walla River samples cluster together, and as expected, cluster regionally with the Touchet River samples. Due to general genetic similarity among steelhead sample groups and the absence of resident trout samples in our study, we are unable to test for the presence of rainbow trout in the Walla Walla River samples.

We report results relevant to concerns about introgression of Lyons Ferry Hatchery fish into the Walla Walla River. First are analyses of Molecular Variance (AMOVA) results and second are individual assignment results. The AMOVA results show that 98.70% of genetic variation observed is present within populations, 0.86% is present among population samples within rivers, and 0.44% is present among rivers. In other words, the Tucannon, Touchet, and Walla Walla Rivers are, in general, closely related, so it is difficult to document the migration of alleles (i.e. introgression). It is unlikely that a complete absence of gene flow exists among these groups. Therefore, the hypothesis to test involves the magnitude of gene flow relative to the time of divergence among these populations. We are unable to distinguish between the competing hypotheses of 1) low gene flow over a long time period or 2) high gene flow over a short time period, due to current genetic similarities among these populations. The assignment of individual steelhead to most-likely population of origin elucidates this issue.

The individual assignment results are shown in Table 9. The Walla Walla sample, which contains mainstem and Mill Creek samples, had the highest self-assignment rate: 56%, the fewest number of individuals assigning to LFH: 1%, and the lowest number of unassigned fish: 27%. This result

suggests Walla Walla River samples are more distinct from LFH than other population samples in this study (i.e. Touchet or Tucannon Rivers). While the assignment results do not quantify introgression (or migration), the results suggest that there is not a large amount of gene flow between Walla Walla River and LFH. If there were a high migration rate from LFH to Walla Walla River, the expectation would be many misassigned individuals from the Walla Walla to the LFH population. Individual assignment results show that the Touchet population sample has a slightly higher misassignment rate to LFH than the Walla Walla sample (Table 9). Regarding Coppei Creek, a lower Touchet River tributary, 86.7% of individuals assigned correctly back to the Touchet population sample (0 to Tucannon, 3 to LFH, 52 to Touchet, and 5 to Walla Walla), which is a higher assignment rate than the overall Touchet sample. There is not strong evidence for hatchery introgression in the Touchet, Coppei, or Walla Walla from LFH based on the individual assignment results.

There is evidence for hatchery introgression in the Tucannon from LFH based on the individual assignment results. The Tucannon sample has a higher proportion of steelhead misassigned to LFH, as compared to Touchet River misassignments to LFH (Table 9). Additionally, Tucannon had the lowest self-assignment rate, and highest proportion of unassigned fish, so there may be demographic factors affecting genetic diversity in the Tucannon River, such as increased numbers of migrants. This is perhaps not surprising given that in the Tucannon River, LFH stock hatchery fish are essentially allowed access all the way to TFH, which results in a large overlap of spawning area. In the Touchet River, hatchery fish tend to come back to the acclimation pond area, so there is less overlap with the majority of the natural spawning area. Ongoing genetic monitoring of natural steelhead populations would be required to document the introgression of LFH steelhead. Introgression can be inferred by observing specific changes in population allele frequencies. The most superior sampling scheme for a genetic monitoring plan would be to collect population samples (approximately N=50 randomly chosen individuals) every year. All samples would not necessarily be genotyped, but all cohorts would be available if needed. A minimum sampling effort for genetic monitoring would be to collect population samples for three consecutive years every ten years. If more detailed monitoring were required, such as following parentage or the calculation of effective population size, more intensive sampling would be required.

Supplemental Question #1 - How do the endemic stocks from the Tucannon, Touchet, and Walla Walla Rivers compare to other steelhead stocks in the Snake or Columbia River basins?

The genetic distance analysis provides results relevant to this question. For multiple samples collected within rivers, the general conclusion is that the genetic relationships among locations remain consistent across sample years (Figures 2 and 3). When the temporally stratified samples were analyzed (Figure 2), there was strong bootstrap support for the Touchet River sample cluster separate from the Tucannon River and LFH samples on the population tree. Additionally, since all the Tucannon River samples cluster together, and all but one of the LFH samples cluster together, there was support for the conclusion that the genetic relationships among populations was consistent from year-to-year. Stated differently, there was a greater genetic affinity among multiple samples from a single location, than among samples collected the same year from different localities. Yet, there were some observations that conflicted with the general conclusion of phylogenetic consistency.

In Figure 2, a well-supported sub-branch within the Touchet River contains the 1999 and 2000 Touchet River samples. This divergent branch may be a case of long-branch-attraction (Hendy and Penny 1989); since those two samples cluster with upper Touchet River tributary samples when additional groups were included in the analysis (Figure 3). It is possible that the small sample sizes collected in 1999 and 2000 imprecisely estimate the actual allele frequencies for these Touchet River samples, which contributed to their placement outside the Touchet River cluster on the trees. Yet, evidence suggests sample size is not the only issue. First, levels of allelic diversity were not atypical for the 1999 and 2000 samples (Table 1), so the sample sizes capture genetic information similar to the other sample collections. Second, when using individual assignment, a higher proportion of fish from the 1999 and 2000 Touchet samples assign to upper Touchet samples and a lower proportion of these same fish assign to Tucannon, when compared to the remaining Touchet River samples (data not shown). Thus, the genetic constitution appears slightly different between the early and late Touchet samples. This slight difference is probably the result of both small sample sizes and the presence of upper Touchet River (north, south and Wolf forks; Table 1) juvenile samples from those years in the dataset. In general, steelhead reproducing in the upper Touchet River are individuals not collected for broodstock that have been allowed to escape trapping in Dayton, so juveniles produced in the upper Touchet would be genetically related to the Touchet River adult samples. Specifically for the 1999 sample year, all Touchet River adults sampled at the Touchet trap were allowed to escape upriver and spawn naturally, and could even be the parents of the juveniles sampled. This relationship is corroborated by results in Table 2, where the 1999 Touchet sample is largely undifferentiated from the juvenile samples. This sampling effect likely altered the relative genetic distances within the phylogenetic tree. Another complicating factor from a different sampling effect is the possible presence of related individuals within juvenile samples, which may have altered allele frequencies from the contributing parental generation. We did not attempt to remove highly related individuals from the juvenile collections and redo the analysis since there was not strong evidence for increased relatedness within the juvenile samples. Relatedness was surveyed by calculating the pairwise relatedness (Queller and Goodnight 1989) for all individuals in the dataset, calculating the arithmetic mean relatedness, then comparing the actual mean with the mean calculated from the null distribution of unrelated individuals. 1000 datasets of N randomly selected genotypes (without replacement) was used to generate the null distribution of relatedness. The p-values for the actual mean pairwise relatedness values by juvenile population are 0.449 for 1999 NFTouJ, 0.958 for 2000 NFTouJ, 0.974 for 1999 SFTouJ, 0.314 for 1999 WFTouJ, 0.108 for 2000 CoppJ, and 0.856 for 2000 RobTouJ. A p-value of 0.95 is significant at alpha=0.05, and a p-value of 0.99 is significant at alpha=0.01. The p-values for the 2000 NFTouJ and 1999 SFTouJ samples suggest some increased relatedness. A non-significant result indicates that individuals are not more related than expected under the null hypothesis. Additionally, whether the juvenile samples were absent (Figure 2) or present (Figure 3), the 1999 and 2000 Touchet River samples were slightly different from the remaining Touchet River samples. This result would not likely be altered by removing possibly related individuals from the juvenile samples, since the expected result would be the shortening of branch lengths on the dendrogram, not a different topology.

To place the genetic distance results into a broader geographic context, we obtained genotype data for a lower Columbia River steelhead population (2000, 2001 Kalama River), and three Grande Ronde juvenile steelhead samples (2000 Rattlesnake Creek, 2000 Cougar Creek, and 2000 Wallowa stock). Moran and Waples (2004) have published population structure information for Snake River

summer steelhead. The 2000 Rattlesnake and 2000 Cougar Creek samples from their study are included in our study. Moran and Waples (2004) also included Tucannon River steelhead in their study (1991, 1992, and 1995), but the sample years are different from our study. Moran and Waples (2004) did not include any populations from the Columbia or Walla Walla Rivers. Our inclusion of the Kalama and Walla Walla River samples provides a wide geographic scope in our study for comparison with the extensive Snake River survey conducted by Moran and Waples (2004). To enhance comparability between this study and Moran and Waples (2004), we present the genetic distance results as a rectangular dendrogram as in Moran and Waples (2004) (Figure 4); however, note that the genetic distance metric used to construct the dendrograms differs between studies. The five replicated samples from both the Tucannon and Touchet Rivers were combined into single samples, as were the two samples from the Kalama River. Our results corroborate the findings of Moran and Waples (2004), with genetic differentiation observed among Tucannon River steelhead and Grande Ronde samples from Rattlesnake Creek, Cougar Creek, and Wallowa stock. We report substantial differentiation among Kalama River, Tucannon River, Touchet River, Walla Walla River, Grande Ronde River, and LFH. The branching structure of the dendrogram (Figure 4) is well supported by bootstrap analysis.

While the F_{ST} metric should not be interpreted as a genetic distance, documenting the amount of total genetic variance attributed to population subdivision is informative (Table 10). The F_{ST} estimate between Kalama and Tucannon samples is 0.038 and the F_{ST} estimate between Kalama and Touchet samples is 0.037. The mean F_{ST} estimate between Kalama and Walla Walla samples is 0.040. These data suggest that the magnitude of divergence among Kalama River steelhead and more interior steelhead populations are similar. The Grande Ronde samples from Rattlesnake Creek and Wallowa stock had a mean pairwise F_{ST} estimate of 0.02 when compared to Tucannon, Touchet, or Walla Walla samples. The Cougar Creek sample was more divergent, with pairwise F_{ST} estimated as 0.043 for Tucannon River, 0.045 for Touchet River, and 0.050 for Walla Walla River. These data suggest substantial differentiation between the Columbia River and Snake River steelhead, and substantial genetic differentiation between Snake River and Walla Walla River steelhead.

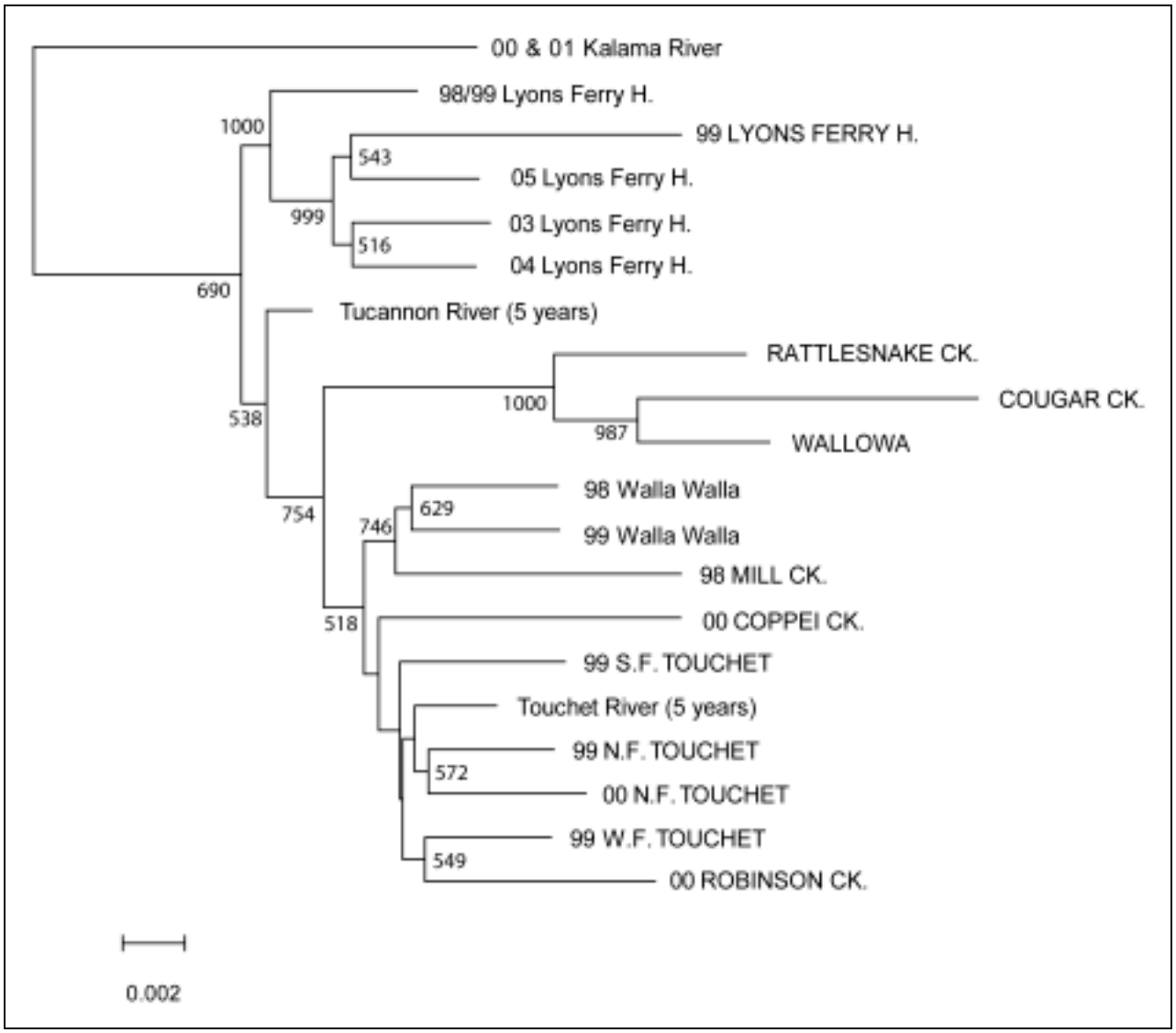

Figure 4. Chord distance tree from steelhead samples from Columbia River, Walla Walla River, and Snake River. Sample labels with all letters capitalized are juvenile samples. Node support numbers are values from bootstrap analysis (1000 bootstraps).

Table 10 Pairwise estimates of F_{ST} for Tucannon, Touchet, LFH, and Walla Walla steelhead compared to Kalama River, Rattlesnake Creek, Cougar Creek, and Wallowa stock.

	Kalama	Rattle	Cougar	Wallowa
Tucannon	0.038	0.017	0.043	0.019
98/99LFH	0.033	0.017	0.039	0.018
99LFH	0.074	0.060	0.087	0.062
03LFH	0.045	0.024	0.054	0.028
04LFH	0.045	0.024	0.055	0.026
05LFH	0.048	0.027	0.054	0.028
Touchet	0.037	0.022	0.045	0.024
99NFTouc	0.040	0.026	0.049	0.027
00NFTouc	0.042	0.027	0.053	0.032
99SFTouc	0.047	0.023	0.049	0.027
99WFTouc	0.040	0.028	0.041	0.028
00Coppei	0.048	0.030	0.052	0.032
00Robins	0.053	0.028	0.050	0.028
98Walla	0.043	0.020	0.056	0.024
99Walla	0.040	0.020	0.048	0.023
98Mill	0.040	0.022	0.052	0.027

Supplemental Question #2 - Given the close similarity between these stocks, how confidently or surely does the data allow us to assign individual fish to the correct location?

For any individual chosen at random, the probability of its genotype belonging to a specific population is based on that population's allele frequencies. Since the steelhead populations in this study are genetically similar, an individual's genotype may have a high likelihood of originating from multiple populations. Of the steelhead sampled from the Tucannon River (N=451) that were assigned based on the LOD > 1 criteria (57%), 29% were correctly assigned back to Tucannon River (Table 7). Of the steelhead sampled from LFH (N=333) that were assigned based on the LOD > 1 criteria (69%), 46% were correctly assigned back to LFH (Table 9). Of the steelhead sampled from the Touchet River (N=987) that were assigned based on the LOD > 1 criteria (70%), 53% were correctly assigned back to Touchet River (Table 9). Of the steelhead sampled from the Walla Walla River (N=177) that were assigned based on the LOD > 1 criteria (73%), 56% were correctly assigned back to Walla Walla River (Table 9). These results suggest that the power to correctly identify an individual steelhead to stock of origin is generally low based on these data; however Walla Walla River exhibits the greatest power and Tucannon River the lowest.

The individual assignment results can be used as a formal power analysis (Table 11). When determining type-1 and type-2 error based on individual assignment, all individuals are assigned (i.e. there is no unassigned fraction based on a LOD criteria). The type-1 error is quantified by observing the number of individuals from a population that misassign to another population. The type-2 error is quantified by observing the number of individuals that are falsely included in a population sample. The power is by definition 1 – type-2 error.

Table 11 Power analysis based on assignment of individual steelhead to stock of origin.

	All samples			Excluding 98/99 LFH and 1999 LFH		
	Type-1	Type-2	Power	Type-1	Type-2	Power
Tucannon River ……	0.670	0.453	0.547	0.615	0.421	0.579
Lyons Ferry Hatchery	0.181	0.423	0.577	0.180	0.420	0.580
Touchet River……	0.175	0.178	0.822	0.168	0.192	0.808
Walla Walla ……	0.376	0.423	0.577	0.370	0.380	0.620

Conservation concerns

The observation of temporal stability of allele frequencies for natural populations and temporal instability at the hatchery suggests that a smaller N_e may exist for the hatchery samples. The BOTTLENECK results corroborate this idea to some degree, as two LFH samples showed heterozygosities in excess of expectations under mutation-drift equilibrium, suggesting a recent reduction in population size. In contrast, when census data is considered for natural and LFH populations, a comparable N_e is expected for natural steelhead and the LFH stock. For brood years between 1998-2006 the harmonic mean of census size was 326.9 for Tucannon River, 336.0 for Touchet River, and 410.0 for LFH (J.D. Bumgarner unpublished data). The census estimate for Tucannon River is likely an underestimate, because for three brood years (1998, 2000, and 2003) the census size was estimated from trapping data not spawning ground surveys. Nevertheless, estimates of N_e calculated for the LFH samples were lower than the natural population samples (Table 5-7). Ratios of the N_e estimated by the temporal method and the harmonic mean of census size are 0.68 for Tucannon River, 0.52 for Touchet River, and 0.35 for LFH. These numbers are consistent with the general thought that N_e is between 0.10 and 0.33 of the estimated census size (Bartley et al. 1992; Waples pers. comm.). Arden and Kapuscinki (2003) found that for 18 brood years of Snow Creek steelhead surveyed, the N_e to N ratio ranged from 0.41 to 0.67, and had an overall harmonic mean of 0.61. We have not yet investigated the possible causes of lower N_e observed for LFH. In general N_e is lower than the N (census size) because of fluctuations in population size, unequal sex ratios, and variance in reproductive success (i.e. the number of offspring produced per individual). The census size of LFH is similar to the natural populations from year to year. Therefore, unequal sex ratios or variance in reproductive success are possible explanations for the slightly lower N_e of LFH.

Since the Tucannon, Touchet, and Walla Walla River summer steelhead are populations of conservation concern, there are management implications to the observations reported. Even though population differentiation was low, in general, the Tucannon, Touchet, and Walla Walla River populations were significantly differentiated, and all groups were differentiated from LFH (Figure 2; Tables 3). Although, the Tucannon River samples were more closely related to the LFH samples, this is likely from 20 years of interbreeding between hatchery and natural fish and not shared

ancestry. The LFH stock was developed primarily from Wells Hatchery Stock (upper Columbia River) and the Wallowa stock (Snake River composite). As such, the LFH stock was not historically similar to either Tucannon River or Touchet River fish. Since that is the case, the Tucannon River steelhead and LFH fish have become similar more rapidly then Touchet River steelhead have, given the genetic distance data (Figures 2 and 3). The difference in convergence rates observed between Tucannon River steelhead and LFH steelhead, as compared to Touchet River and LFH, is likely due to differences in the magnitude of recent gene flow (i.e. hatchery introgression). Ecological and genetic data support this supposition. First, juveniles from LFH are released into the Tucannon, Touchet, and Walla Walla Rivers. Yet, historically there was more opportunity for gene flow in the Tucannon River, since hatchery juveniles were released in the vicinity of spawning habitat for natural Tucannon River steelhead and thus may have returned to the spawning area of natural steelhead (Bumgarner et al. 2003). Furthermore, hatchery origin adults that are not brought into the hatchery for spawning are left in the stream to increase sport-fishing opportunities within the Tucannon River. In contrast, LFH stock juveniles are released at the lower end of spawning areas for natural steelhead in both the Touchet and Walla Walla River (Bumgarner et al. 2003). Second, genetic distance and individual assignment results were consistent with differential gene flow between all three natural steelhead populations studied and LFH stock (Figure 3; Table 9). The Tucannon River natural adults were similar to LFH and the Touchet River natural steelhead were divergent from Tucannon River, LFH, and other Walla Walla River samples included in the study. Narum et al. (2004) also observed differentiation between Touchet River and Walla Walla River populations. Figure 3 shows the Touchet River samples as a distinct branch and reliably places that branch between the samples from the Snake River and Walla Walla River. Individual assignment results show that Touchet samples were more distinct from LFH than Tucannon samples, as a higher proportion of Tucannon River fish were misassigned to LFH, as compared to Touchet River misassignments to LFH (Table 9). Additionally, Tucannon had the lowest self-assignment rate, and highest proportion of unassigned fish. While the Tucannon, Touchet and Walla Walla Rivers are all distinct from each other and LFH, we conclude that the collective data provides evidence for hatchery introgression in the Tucannon River, but not the Touchet or Walla Walla rivers. If LFH releases continue in the Tucannon, Touchet, and Walla Walla Rivers, it will be important to continue monitoring the populations for changes in genetic composition, effective population sizes, and estimates of gene flow. The most superior sampling scheme for a genetic monitoring plan would be to collect population samples (approximately N=50 randomly chosen individuals) every year. The age data and tissue should be archived for future analysis.

Acknowledgements

Samples:
We would like to thank Jon Germond and Tim Bailey, Oregon Department of Fish and Wildlife, and Larry Boe, U.S. Forest Service, for collecting steelhead from the Upper Walla Walla River and upper Mill Creek, respectively.
We would like to thank Dave Karl, Jeremy Trump, Mike Gembala, Jerry Dedloff, and Lance Ross, Washington Department of Fish and Wildlife.

Laboratory:
We would like to thank Cherril Bowman, Norm Switzler, and Judy Morgan for laboratory analysis

Reviews:
We would like to thank Denise Hawkins and Ken Warheit for helpful reviews.

Funding:
Funding for this project was provided by the Lower Snake River Compensation Plan Program, Bonneville Power Administration, and WDFW through the Washington State General Fund.

Literature Cited

Arden WR and Kapuscinski AR (2003) Demographic and genetic estimates of effective population size (N_e) reveals genetic compensation in steelhead trout. Molecular Ecology 12: 35-49

Banks MA, Blouin MS, Baldwin BA et al (1999) Isolation and inheritance of novel microsatellites in chinook salmon (Oncorhynchus tschawytscha). Journal of Heredity 90:281-288.

Banks MA, Rashbrook VK, Calavetta MJ et al (2000) Analysis of microsatellite DNA resolves genetic structure and diversity of chinook salmon (Oncorhynchus tshawytscha) in California's Central Valley. Canadian Journal of Fisheries and Aquatic Sciences 57:915-927.

Bartley D, Bentley B, Brodziak J et al (1992) Geographic variation in population genetic structure of chinook salmon from California and Oregon. Fishery Bulletin, U.S. 90:77-100.

Belkhir K, Borsa P, Chikhi L et al (2001) 1996-2001 GENETIX 4.02, logiciel sous Windows TM pour la Génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Montpellier (France).

Busack C, Pearsons T, Knudsen C et al (1997) Yakima Fisheries Project Spring Chinook Supplementation Monitoring Plan. U. S. Department of Energy, Bonneville Power Administration, BPA Report DOE/BP-64878-1, Pp. 176.

Busby PJ, Wainwright TC, Bryant GJ et al (1997) Status Review of West Coast Steelhead from Washington, Idaho, Oregon, and California. U.S. Dept. of Commerce, NMFS-NWFSC-27, Pp. 275.

Bumgarner J, Small MP, Ross L et al (2003) Lyons Ferry Complex Hatchery Evaluation: summer steelhead and trout report 2001 and 2002 run years. Washington Department Fish and Wildlife, Annual Report Number: FPA03-15.

Bumgarner JD, Dedloff J, Herr M (2006) Lyons Ferry Complex Hatchery Evaluation: Summer Steelhead Annual Report 2004 Run Year to USFWS Lower Snake River Compensation Plan Office, Boise, ID. FPA 06-01. 46pp.

Campton, DE (1987) Natural hybridisation and introgression in fishes: methods of detection and genetic interpretations. In: Ryman, N and Utter, F (Eds.) Population genetics and fisheries management. Washington Sea Grant Program, University of Washington Press, Seattle, USA.

Carlsson J, Nilsson J (2000) Population genetic structure of brown trout (Salmo trutta L.) within a northern boreal forest stream. Hereditas 132:173–181.

Cavalli-Sforza LL, Edwards AWF (1967) Phylogenetic analysis: models and estimation procedures. Evolution 32:550-570.

Estoup A, Rousset F, Michalakis Y et al (1998) Comparative analysis of microsatellite and allozyme markers: a case study investigating microgeographic differentiation in brown trout (Salmo trutta). Molecular Ecology 7:339-353.

Excoffier L, Smouse P, and Quattro J (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics 131:479-491.

Excoffier L, Laval G, and Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47-50.

Felsenstein J (2005) PHYLIP (Phylogeny Inference Package). Department of Genome Sciences, University of Washington, Seattle.

Frankham R, Ballou JD, Briscoe DA (2002). Introduction to Conservation Genetics, Cambridge University Press, Cambridge, UK.

Goudet J (1995) FSTAT (Version 1.2): A computer program to calculate F-statistics. Journal of Heredity 86: 485-486.

Hansen MM, Ruzzante DE, Nielsen E et al (2002) Long-term effective population sizes, temporal stability of genetic composition and potential for local adaptation in anadromous brown trout (Salmo trutta) populations. Molecular Ecology 11:2523-2535.

Hedgecock D, Chow V, Waples RS (1992) Effective population numbers of shellfish broodstocks estimated from temporal variance in allelic frequencies. Aquaculture 108:215–232.

Hedrick PW, (1983) Genetics of Populations, Science Books International, Boston

Hendy, MD and Penny, D. (1989) A framework for the quantitative study of evolutionary trees. Systematic Zoology 38: 297–309.

Jensen LF, Hansen MM, Carlsson J et al (2005) Spatial and temporal genetic differentiation and effective population size of brown trout (Salmo trutta, L.) in small Danish rivers. Conservation Genetics 6:615-621.

Laikre L, Jarvi T, Johansson L et al (2002) Spatial and temporal population structure of sea trout at the Island of Gotland, Sweden, delineated from mitochondrial DNA. Journal of Fish Biology 60:49–71.

Lessios HA, Weinberg JR, Starczak VR (1994) Temporal Variation in Populations of the Marine Isopod Excirolana: How Stable are Gene Frequencies and Morphology? Evolution 48:549-563.

Moran P, Waples RS (2004) Monitor and Evaluate the Genetic Characteristics of Supplemented Salmon and Steelhead, 1999-2003 Progress Report, Project No. 198909600. U. S. Department of Energy, Bonneville Power Administration, BPA Report DOE/BP-00015834-1.

Morris DB, Richard KR, Wright JM (1996) Microsatellites from rainbow trout (Oncorhynchus mykiss) and their use for genetic study of salmonids. Canadian Journal of Fisheries & Aquatic Sciences 53:120-126.

Narum SR, Contor C, Talbot A, et al (2004) Genetic divergence of sympatric resident and anadromous forms of Oncorhynchus mykiss in the Walla Walla River, U.S.A. Journal of Fish Biology 65: 471-488.

Nei M (1987) Molecular Evolutionary Genetics, Columbia University Press, New York.

Nei M, Tajima F (1981) Genetic drift and estimation of effective population size. Genetics 98: 625–640.

Nelson RJ, Beacham TD (1999) Isolation and cross species amplification of microsatellite loci useful for study of Pacific salmon. Animal Genetics 30:228-229.

Nielsen EE, Hansen MM, Loeschcke V (1999) Genetic variation in time and space: microsatellite analysis of extinct and extant populations of Atlantic salmon. Evolution 53:261–268.

Olsen JB, Wilson SL, Kretschmer EJ et al (2000) Characterization of 14 tetranucleotide microsatellite loci derived from sockeye salmon. Molecular Ecology 9:2185-2187.

Østergaard S, Hansen MM, Loeschcke V et al (2003) Long-term temporal changes of genetic composition in brown trout (Salmo trutta L.) populations inhabiting an unstable environment. Molecular Ecology 12:3123–3135.

Paetkau D, Slade R, Burden M et al (2004) Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. Molecular Ecology 13:55-65.

Palm S, Laikre L, Jorde PE, et al (2003) Effective population size and temporal genetic change in stream resident brown trout (Salmo trutta, L.). Conservation Genetics 4:249-264.

Park SDE (2001) Trypanotolerance in West African Cattle and the Population Genetic Effects of Selection. University of Dublin.

Peel D, Ovenden JR, Peel SL (2004) NeEstimator: software for estimating effective population size, Version 1.3. Queensland Government, Department of Primary Industries and Fisheries

Piry S, Alapetite A, Cornuet JM, et al (2004) GENECLASS2: A software for genetic assignment and first-generation migrant detection. Journal of Heredity 95:536-539.

Piry S, Luikart G, Cornuet JM (1998) BOTTLENECK, a program for detecting recent population effective population size reductions from allele frequency data, Available from http://www.ensam.inra.fr.URLB.

Pollack E (1983) A new method for estimating the effective population size from allele frequency changes. Genetics 104:531-548

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. Evolution 43(2):258-275.

Rannala B, Mountain JL (1997) Detecting immigration by using multilocus genotypes. Proceedings of the National Academy of Sciences 94:9197-9201.

Raymond M, Rousset F (1995) GENEPOP (Version-1.2) - Population-Genetics Software for Exact Tests and Ecumenicism. Journal of Heredity 86:248-249.

Rexroad CE, Coleman RL, Martin AM et al (2001) Thirty-five polymorphic microsatellite markers for rainbow trout (Oncorhynchus mykiss). Animal Genetics 32:317-319.

Ryman N, Laikre L (1991) Effects of supportive breeding on the genetically effective population size. Conservation Biology 5:325-329.

Small MP, Beacham TD, Withler RE et al (1998) Discriminating coho salmon (Oncorhynchus kisutch) populations within the Fraser River, British Columbia. Molecular Ecology 7:141-155.

Sokal RR, Rohlf FJ (2003) Biometry, W. H. Freeman and Company, New York.

Spies IB, Brasier DJ, O'Reilly PT et al (2005) Development and characterization of novel tetra-, tri-, and dinucleotide microsatellite markers in rainbow trout (Oncorhynchus mykiss). Molecular Ecology 5:278-281.

Tajima F (1992) Statistical method for estimating the effective population size in Pacific salmon. Journal of Heredity 83: 309-311

Tessier N, Bernatchez L (1999) Stability of population structure and genetic diversity across generations assessed by microsatellites among sympatric populations of landlocked Atlantic salmon (Salmo salar L.). Molecular Ecology 8:169-179.

Waples RS (1990) Conservation genetics of Pacific salmon: III. estimating effective population size. Journal of Heredity, 81: 277-289

Waples R (1991) Genetic methods for estimating the effective size of cetacean populations. Report of the International Whaling Commission, Special issue 13, Pp. 279-300.

Waples RS, Johnson OW, Aebersold PB et al (1993) A genetic monitoring and evaluation program for supplemented populations of salmon and steelhead in the Snake River Basin. Annual report. Bonneville Power Administration. DE-A179-89BP0091. Pp. 41

Waples RS (1998) Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. Heredity 89:438-450.

Waples R (2005) Genetic estimates of contemporary effective population size: to what time periods do the estimates apply? Molecular Ecology 14:3335-3352.

Waples RS, Masuda M, and Pella J (2007) SALMONNb: a program for computing cohort-specific effective population sizes (N_b) in Pacific salmon and other semelparous species using the temporal method. Molecular Ecology Notes 7: 21-24

Weir BS (1979) Inferences about linkage disequilibrium. Biometrics 35:235-254.

Weir BS, Cockerham CC (1984) Estimating F-Statistics for the Analysis of Population-Structure. Evolution 38:1358-1370.

Weir B, Hill W (1980) Effect of mating structure on variation in linkage disequilibrium. Genetics 95:477-488.

APPENDIX 1

Allele size range (S), Total number of alleles (A_T), sample size (N), observed heterozygosity (H_O), unbiased heterozygosity (H_E) by locus for multi-year samples of summer steelhead from Tucannon River, Lyons Ferry Hatchery, and Touchet River

		Tucannon River						
Locus		98/99	2000	2001	2002	2003	2004	2005
One-102								
	S	188-285	188-289	188-261	188-285	188-290	188-285	188-285
	A_T	17	21	19	20	22	22	23
	N	36	45	51	45	85	69	127
	H_O	0.778	0.909	0.843	0.842	0.941	0.971	0.921
	H_E	0.901	0.919	0.919	0.924	0.912	0.927	0.917
Ots-100								
	S	168-203	168-215	168-215	168-219	168-215	168-215	160-215
	A_T	10	15	13	19	12	16	17
	N	36	45	51	45	85	69	127
	H_O	0.778	0.844	0.824	0.864	0.795	0.826	0.787
	H_E	0.808	0.833	0.825	0.848	0.816	0.843	0.848
One-114								
	S	189-260	181-272	189-280	181-280	189-280	189-281	189-345
	A_T	17	21	17	22	21	19	22
	N	36	45	51	45	85	69	127
	H_O	0.917	0.864	0.961	0.932	0.940	0.891	0.960
H_E	0.928	0.927	0.922	0.947	0.927	0.932	0.929	
One-101								
	S	119-222	119-222	119-166	119-198	119-235	119-230	119-254
	A_T	3	6	4	4	6	9	8
	N	36	45	51	45	85	69	127
	H_O	0.314	0.405	0.392	0.432	0.482	0.448	0.405
H_E	0.312	0.449	0.393	0.514	0.421	0.461	0.374	
One-108								
	S	169-265	169-261	169-269	169-257	169-261	169-249	169-261
	A_T	17	17	17	18	18	19	21
	N	36	45	51	45	85	69	127
	H_O	0.771	0.833	0.804	0.762	0.777	0.833	0.873
H_E	0.918	0.926	0.932	0.923	0.923	0.907	0.921	

Ots-103

	S	74-90	56-90	60-90	74-94	74-90	60-90	60-90
	A_T	4	6	6	5	4	5	6
	N	36	45	51	45	85	69	127
	H_O	0.314	0.262	0.300	0.273	0.294	0.169	0.238
H_E	0.347	0.260	0.306	0.251	0.275	0.161	0.240	

Ots-1

	S	162-245	164-247	162-245	162-247	164-245	158-247	162-249
	A_T	13	12	11	11	13	16	17
	N	36	45	51	45	85	69	127
	H_O	0.639	0.591	0.740	0.861	0.747	0.725	0.646
	H_E	0.829	0.826	0.811	0.841	0.810	0.836	0.836

Omy-77

	S	99-134	101-134	99-134	97-140	99-147	99-147	99-147
	A_T	14	16	16	19	17	18	19
	N	36	45	51	45	85	69	127
	H_O	0.889	0.711	0.776	0.886	0.747	0.818	0.819
H_E	0.894	0.894	0.912	0.899	0.891	0.922	0.908	

Ots-3M

	S	134-145	134-147	128-147	132-156	132-156	128-145	134-156
	A_T	6	7	8	8	8	8	7
	N	36	45	51	45	85	69	127
	H_O	0.667	0.756	0.766	0.636	0.750	0.696	0.701
	H_E	0.735	0.723	0.746	0.741	0.713	0.717	0.728

Omy-1001

	S	181-224	167-224	175-224	175-224	167-224	162-224	167-228
	A_T	14	20	18	16	20	19	26
	N	36	45	51	45	85	69	127
	H_O	0.889	0.889	0.922	1.000	0.868	0.925	0.929
	H_E	0.905	0.917	0.918	0.929	0.921	0.922	0.932

Omm-1128

	S	206-337	211-345	211-365	227-357	223-388	207-357	207-373
	A_T	28	20	26	24	29	30	34
	N	36	45	51	45	85	69	127
	H_O	0.857	0.775	0.896	0.906	0.817	0.853	0.889
H_E	0.959	0.940	0.953	0.950	0.955	0.949	0.948	

Omm-1130

	S	200-372	197-387	197-379	200-341	197-379	197-368	197-379
	A_T	23	31	30	26	36	29	44
	N	36	45	51	45	85	69	127

H_O	0.944	0.978	0.922	0.884	0.964	0.927	0.969
H_E	0.946	0.961	0.956	0.958	0.955	0.953	0.961
Omm-1070							
S	164-369	164-384	164-354	164-330	172-369	164-358	164-384
A_T	25	30	25	22	34	30	37
N	36	45	51	45	85	69	127
H_O	0.944	0.933	0.902	0.886	0.817	0.809	0.832
H_E	0.956	0.963	0.947	0.948	0.957	0.945	0.955
Omy-1011							
S	151-203	138-210	138-249	138-206	138-206	138-214	138-206
A_T	14	16	19	13	16	16	17
N	36	45	51	45	85	69	127
H_O	1.000	0.844	0.880	0.839	0.868	0.853	0.873
H_E	0.885	0.897	0.900	0.890	0.874	0.902	0.908

	Lyons Ferry Hatchery				
Locus	98/99	1999	2003	2004	2005
One-102					
S	188-285	188-289	188-285	188-290	188-290
A_T	19	18	20	22	20
N	45	48	100	100	100
H_O	0.884	0.875	0.860	0.940	0.910
H_E	0.924	0.926	0.916	0.919	0.907
Ots-100					
S	168-215	168-203	168-215	168-203	168-215
A_T	14	12	12	11	14
N	45	48	100	100	100
H_O	0.829	0.830	0.727	0.800	0.830
H_E	0.854	0.846	0.768	0.828	0.844
One-114					
S	181-280	189-280	177-280	189-276	189-281
A_T	20	18	22	18	21
N	45	48	100	100	100
H_O	0.905	0.875	0.878	0.869	0.960
H_E	0.938	0.931	0.931	0.919	0.933
One-101					
S	119-131	119-178	119-230	119-254	119-230

	A_T	3	3	5	7	4
	N	45	48	100	100	100
	H_O	0.415	0.319	0.392	0.340	0.290
	H_E	0.397	0.394	0.405	0.363	0.324
One-108						
	S	169-245	177-245	169-245	169-245	169-244
	A_T	18	15	17	16	17
	N	45	48	100	100	100
	H_O	0.886	0.830	0.792	0.788	0.880
	H_E	0.930	0.889	0.892	0.908	0.906
Ots-103						
	S	74-94	65-90	60-90	78-90	78-90
	A_T	5	4	6	4	4
	N	45	48	100	100	100
	H_O	0.326	0.174	0.250	0.181	0.380
	H_E	0.290	0.165	0.231	0.207	0.336
Ots-1						
	S	122-247	120-247	162-247	162-247	162-247
	A_T	12	13	12	14	12
	N	45	48	100	100	100
	H_O	0.857	0.702	0.687	0.776	0.680
	H_E	0.850	0.860	0.852	0.849	0.833
Omy-77						
	S	99-138	101-134	99-134	103-147	103-134
	A_T	16	12	15	16	12
	N	45	48	100	100	100
	H_O	0.844	0.766	0.849	0.814	0.730
	H_E	0.915	0.873	0.895	0.917	0.887
Ots-3M						
	S	132-145	132-145	132-156	132-156	132-156
	A_T	7	7	8	8	8
	N	45	48	100	100	100
	H_O	0.698	0.617	0.753	0.794	0.830
	H_E	0.750	0.748	0.758	0.758	0.799
Omy-1001						
	S	167-224	167-224	167-224	175-224	175-224
	A_T	19	17	17	18	18
	N	45	48	100	100	100
	H_O	0.875	0.936	0.901	0.950	0.940
	H_E	0.928	0.884	0.911	0.914	0.914

Omm-1128					
S	211-341	231-300	211-350	211-365	211-350
A_T	24	7	25	29	27
N	45	48	100	100	100
H_O	0.974	0.174	0.935	0.930	0.900
H_E	0.942	0.243	0.928	0.935	0.935
Omm-1130					
S	197-383	197-368	197-304	197-383	197-379
A_T	27	25	25	27	30
N	45	48	100	100	100
H_O	0.900	0.938	0.794	0.939	0.930
H_E	0.953	0.932	0.933	0.943	0.938
Omm-1070					
S	164-369	164-334	164-322	164-384	164-384
A_T	26	20	22	31	29
N	45	48	100	100	100
H_O	0.889	0.875	0.798	0.849	0.930
H_E	0.949	0.924	0.927	0.932	0.944
Omy-1011					
S	138-199	138-230	138-203	138-199	138-203
A_T	14	15	15	14	15
N	45	48	100	100	100
H_O	0.857	0.915	0.923	0.869	0.910
H_E	0.912	0.920	0.895	0.894	0.899

	Touchet River						
Locus	1999	2000	2001	2002	2003	2004	2005
One-102							
S	192-253	188-277	188-285	188-285	188-277	188-277	188-285
A_T	14	15	23	22	21	21	22
N	33	30	116	85	73	96	75
H_O	0.893	0.900	0.887	0.868	0.940	0.925	0.880
H_E	0.897	0.911	0.911	0.911	0.929	0.913	0.897
Ots-100							
S	168-211	168-205	160-209	168-215	168-215	168-211	160-224
A_T	11	11	13	15	13	13	16
N	33	30	116	85	73	96	75
H_O	0.857	0.862	0.868	0.840	0.868	0.813	0.867

H_E	0.871	0.860	0.854	0.856	0.860	0.822	0.868
One-114							
S	189-280	189-256	185-272	181-260	189-260	185-281	189-272
A_T	20	16	21	19	18	22	19
N	33	30	116	85	73	96	75
H_O	0.893	0.931	0.876	0.904	0.846	0.883	0.946
H_E	0.923	0.904	0.907	0.910	0.897	0.916	0.918
One-101							
S	119-127	116-127	119-239	119-235	119-239	119-254	119-262
A_T	2	4	8	5	5	6	9
N	33	30	116	85	73	96	75
H_O	0.394	0.300	0.489	0.381	0.386	0.458	0.514
H_E	0.416	0.606	0.521	0.420	0.457	0.494	0.562
One-108							
S	169-269	181-257	169-269	169-269	169-261	169-317	169-267
A_T	15	13	21	22	17	19	20
N	33	30	116	85	73	96	75
H_O	0.849	0.800	0.770	0.918	0.843	0.819	0.800
H_E	0.895	0.874	0.883	0.917	0.891	0.881	0.905
Ots-103							
S	56-90	82-90	60-90	60-90	60-90	60-90	60-90
A_T	7	3	5	5	5	5	5
N	3	30	116	85	73	96	75
H_O	0.333	0.200	0.228	0.262	0.167	0.263	0.247
H_E	0.303	0.188	0.243	0.258	0.158	0.266	0.248
Ots-1							
S	164-247	158-245	164-247	158-245	164-247	164-256	158-247
A_T	10	10	11	14	11	13	12
N	3	30	116	85	73	96	75
H_O	0.594	0.767	0.711	0.812	0.753	0.821	0.773
H_E	0.844	0.834	0.848	0.869	0.859	0.858	0.853
Omy-77							
S	99-134	99-147	103-134	99-147	99-134	97-134	99-147
A_T	14	15	15	15	14	18	16
N	3	30	116	85	73	96	75
H_O	0.700	0.828	0.830	0.777	0.781	0.844	0.853
H_E	0.909	0.900	0.877	0.887	0.882	0.902	0.897
Ots-3M							
S	134-156	132-145	136-147	132-147	134-147	134-145	134-145

A_T	6	7	6	8	7	6	6
N	3	30	116	85	73	96	75
H_O	0.719	0.571	0.705	0.729	0.603	0.635	0.667
H_E	0.756	0.655	0.659	0.702	0.668	0.702	0.702
Omy-1001							
S	167-216	167-216	167-228	167-228	167-228	167-224	167-228
A_T	14	15	20	17	20	18	18
N	3	30	116	85	73	96	75
H_O	0.906	0.897	0.948	0.916	0.932	0.874	0.947
H_E	0.908	0.910	0.921	0.907	0.917	0.919	0.918
Omm-1128							
S	223-357	223-329	206-337	215-365	215-373	207-388	207-369
A_T	27	25	31	25	27	33	32
N	3	30	116	85	73	96	75
H_O	0.879	0.931	0.904	0.847	0.836	0.915	0.878
H_E	0.962	0.967	0.946	0.936	0.947	0.950	0.947
Omm-1130							
S	197-376	197-376	197-376	197-379	197-379	197-379	197-383
A_T	30	25	35	35	32	34	35
N	3	30	116	85	73	96	75
H_O	0.970	0.933	0.917	0.940	0.890	0.926	0.920
H_E	0.968	0.949	0.958	0.955	0.949	0.964	0.963
Omm-1070							
S	164-334	164-334	164-322	164-354	164-330	164-354	164-354
A_T	26	24	27	27	29	29	28
N	3	30	116	85	73	96	75
H_O	0.788	1.000	0.759	0.732	0.729	0.830	0.867
H_E	0.931	0.949	0.937	0.940	0.946	0.943	0.943
Omy-1011							
S	147-230	138-203	138-210	138-203	138-206	134-210	138-210
A_T	14	14	17	16	16	19	17
N	3	30	116	85	73	96	75
H_O	0.939	0.867	0.872	0.963	0.900	0.895	0.901
H_E	0.890	0.864	0.889	0.889	0.881	0.899	0.900